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Viral Dynamics in HIV-1 Infection

Review

Diana Finzi and Robert F. Siliciano
Department of Medicine
The Johns Hopkins University School of Medicine
Baltimore, Maryland 21205

A great deal of progress has been made in understanding the molecular biology of HIV-1 replication (see reviews by Cullen [1998], Chan and Kim [1998], and Littman [1998] in this issue of *Cell*). Nevertheless, critical aspects of AIDS pathogenesis remain to be elucidated. For example, it is still unclear how HIV-1 infection induces $CD4^+$ T cell depletion, which is the central pathophysiologic feature of the disease. It is also uncertain how the virus persists in the presence of vigorous immune responses. Recently, important insights into these complex aspects of the host–pathogen interaction in HIV-1 infection have come from a surprisingly simple approach, the direct measurement of circulating levels of free virus. The development of rapid, sensitive, and accurate methods for quantitating virus particles in the blood has greatly facilitated the study of AIDS pathogenesis and the management of patients with HIV-1 infection. Quantitative competitive RT-PCR is carried out on blood and other biological fluids to detect genomic viral RNA, which is present at two copies per virion (Piatak et al., 1993). Because the rate of clearance of free virus from the blood is largely independent of stage of disease and other factors, the level of viral RNA in the plasma reflects the rate of virus production. Recent studies suggest that the half-life of free virus particles is extremely short, on the order of minutes to hours (Perelson et al., 1996). Thus, the steady-state level of viral RNA genomes in the blood reflects very recent virus production. As a result, physicians and investigators in this field have a tool that is available for few other infectious diseases, a real-time measure of the rate of replication of the pathogen in the host. Analysis of dynamic changes in the level of plasma virus at different stages of disease and in response to antiretroviral therapy has provided some startling new insights into the mechanism of $CD4^+$ T cell depletion, the reasons for drug failure, the nature of viral reservoirs, and the intriguing possibility that prolonged therapy may lead to virus eradication. The measurement of viral dynamics in vivo is a new area of study, made possible by the availability of assays for free virus and of antiretroviral drugs that perturb the steady-state equilibrium between virus production and virus clearance. It is likely that lessons learned will have broad-reaching influence on the study of other infectious diseases. This review will discuss recent advances in the study of viral dynamics, focusing on the possibility of HIV-1 eradication with antiretroviral therapy.

The Steady State

One important and incontrovertible principle to emerge from measurements of plasma virus levels in HIV-1 infection is that viral replication continues throughout the disease, even during the prolonged asymptomatic phase between primary infection and the development of AIDS (Coombs et al., 1989; Ho et al., 1989; Piatak et al., 1993). Primary HIV-1 infection is characterized by extremely high levels of plasma virus (Daar et al., 1991). Values in excess of 10^6 copies of RNA/ml are commonly seen. As the immune response to HIV-1 develops, plasma virus levels fall to lower steady-state values that vary in different individuals and that are predictive of the rate of disease progression (Mellors et al., 1996). In untreated asymptomatic patients, the plasma HIV-1 RNA levels are typically in the range of 10^3–10^6 copies/ml in blood. Titers of infectious virus are several orders of magnitude lower, indicating that much of this plasma virus is defective, decayed, or neutralized.

During the asymptomatic phase of the infection, the level of plasma HIV-1 RNA is reasonably stable in a given individual in the short run (days to weeks). This reflects a quasi–steady state in which virus production equals virus clearance. The equilibrium has been formalized by Perelson and colleagues in the form of the equation $P = cV$ where P is the viral production rate, c is the viral clearance rate constant, and V is the number of plasma virions (Ho et al., 1995; Perelson et al., 1996, 1997a). At a first approximation, the dynamics underlying this equilibrium between virus production and virus clearance can be represented as:

$$dV/dt = N\delta T^* - cV$$

in which $N\delta T^*$, the virus production term, is the product of the number of new virions produced per infected cell (N), the rate of loss of productively infected cells (δ), and the number of productively infected cells (T^*). At steady state, $dV/dt = 0$ and $N\delta T^* = cV$. As is discussed below, perturbation of this equilibrium by treatment with antiretroviral drugs has given important information about the rates of virus production and the rates of clearance of free virus particles (c) and of productively infected cells (δ). Perelson et al. have also constructed more elaborate models that take into account virus production by different cell types with different rates of clearance. These models have proven very useful in the analysis of viral reservoirs in infected individuals (Perelson et al., 1996, 1997a). Before considering these models of viral dynamics, it is worth reviewing in a qualitative sense where the plasma virus comes from and what happens to it.

Current evidence favors the idea that most of the plasma virus comes from recently infected $CD4^+$ lymphoblasts in the peripheral lymphoid tissues. The infection of $CD4^+$ T cells by HIV-1 is best considered in the context of the normal physiology of T cell activation (Figure 1). Uninfected $CD4^+$ T cells emerge from the thymus as naive cells and circulate until they encounter antigen. They then undergo blast transformation and begin to proliferate. Some of the cells survive and return to a G_0 resting state in which they persist as memory cells able to respond again to subsequent encounters with the same antigen. Memory T cells are distinguishable from naïve cells based on differential expression of adhesion molecules and altered triggering thresholds.

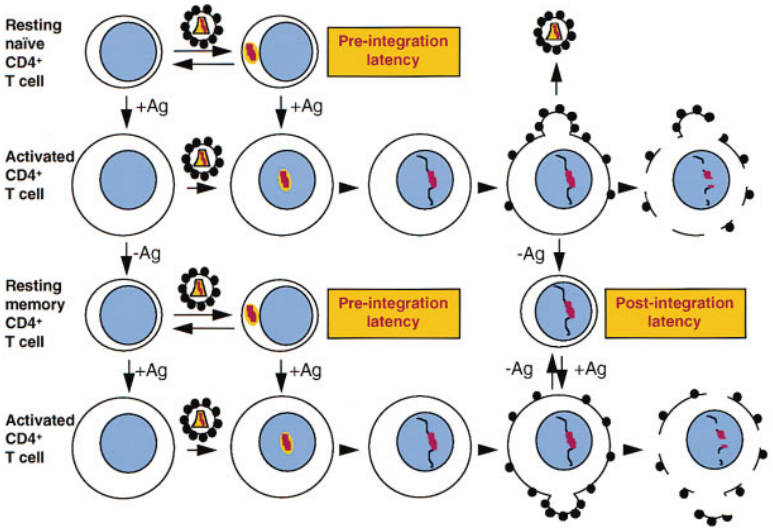

Figure 1. Cellular Dynamics of HIV-1 Infection of CD4$^+$ T Cells

Successive steps in the life cycle of the virus are indicated by horizontal arrows. Transitions between resting (small) and activated (large) CD4$^+$ T cells are illustrated by vertical arrows. X4 isolates of HIV-1 can infect resting and activated CD4$^+$ T cells, but replication does not occur in resting cells due to a block prior to the stage of nuclear import of the preintegration complex containing the reverse transcribed HIV-1 DNA. R5 isolates can infect activated CD4$^+$ T cells, but may infect only the subset of resting CD4$^+$ T cells that express sufficient amounts of CCR5. Note that resting cells with unintegrated HIV-1 DNA are likely to represent a relatively labile reservoir for the virus (preintegration latency). Productive infection requires antigen-driven activation of recently infected resting CD4$^+$ cells or, more commonly, direct infection of antigen-activated CD4$^+$ T cells. Productively infected cells generally die within a few days from cytopathic effects of the infection or host cytolytic effector mechanisms, but some infected lymphoblasts survive long enough to go back to a resting state, thereby establishing a stable latent reservoir of resting memory CD4$^+$ T cells with integrated HIV-1 DNA (postintegration latency).

T cell tropic (X4) HIV-1 isolates can infect CD4$^+$ T cells in all of these stages. Macrophage tropic (R5) HIV-1 isolates can infect activated CD4$^+$ T cells but may not infect resting cells readily because of the low expression of CCR5. In any event, the infection does not progress in resting cells due to a block prior to the stage of nuclear import of the preintegration complex containing the reverse transcribed HIV-1 DNA. Resting cells with unintegrated HIV-1 DNA may represent a latent reservoir for the virus in the sense that if these cells are activated by antigen before the preintegration complex decays, then the subsequent steps of nuclear import, integration, virus gene expression and virus production occur (Zack et al., 1990; Bukrinsky et al., 1991, 1992; Spina et al., 1995). The major pathway to productive infection probably involves direct infection of activated CD4$^+$ T cells. These infected lymphoblasts are highly permissive for viral replication and rapidly begin to produce virus. However, infected CD4$^+$ lymphoblasts die quickly ($t_{1/2} \sim$ 1 day, see below) due to viral cytopathic effects and/or host cytolytic effector mechanisms. A small fraction of the productively infected cells may survive long enough to revert back to a resting memory state in much the same way that noninfected CD4$^+$ lymphoblasts do. The resulting memory cells carry an integrated copy of the HIV-1 genome. Because of the absence of activation-dependent host transcription factors and insufficient levels of positive regulatory viral factors such as tat and rev, the integrated provirus in resting memory cells remains latent until the memory cell is reactivated by antigen (Folks et al., 1989; Pomerantz et al., 1990, 1992; Garcia-Blanco and Cullen, 1991; Seshamma et al., 1992). These resting memory cells thus represent a second type of latent reservoir for HIV-1, one with a great potential for stability resulting from the integrated nature of the latent provirus and the long life span of the memory cells harboring the latent provirus.

It is also important to appreciate the pool sizes of the different cells represented in Figure 1. In an uninfected individual, most T cells at any given time are in a resting state, with an approximately equal fraction of cells in the naïve and memory pools. In infected individuals, the proportion of activated cells increases. The fraction of CD4$^+$ T cells that are infected has been controversial, but recent evidence suggests that for both resting and activated CD4$^+$ T cells, the fraction of cells with stably integrated, replication-competent provirus is very small ($<$0.1%) (Chun et al., 1997a). Among resting CD4$^+$ T cells, a higher fraction of cells (1%) may harbor unintegrated HIV-1 DNA (Bukrinsky et al., 1991; Chun et al., 1997a).

The dynamics of infection are somewhat different in the case of cells of the monocyte-macrophage lineage, the other major cell lineage that is susceptible to infection in vivo (Ho et al., 1986; Gartner et al., 1986; Nicolson et al., 1986). Circulating monocytes, the precursors of tissue macrophages, do not show a significant level of infection as assessed by DNA PCR, indicating that infection may occur after the monocytes have left the circulation and differentiated into macrophages (Schnittman et al., 1989). In in vitro infections, macrophages do not succumb to the cytopathic effects of HIV-1 infection. This observation has led to the suggestion that infected macrophages may represent a major reservoir for the virus in vivo. As is the case with CD4$^+$ T cells, only a minute fraction of the macrophage pool carry integrated provirus at any given time (Chun et al., 1997a). Macrophages play an important role in the presentation of antigens to CD4$^+$ T cells, and it is possible that infected macrophages may transmit the infection to CD4$^+$ T cells during antigen presentation. The same is true of bone marrow–derived dendritic cells, which are highly specialized for antigen presentation (Cameron et al., 1992; Pope et al., 1994). The frequency and half-life of infected dendritic cells in vivo is unknown.

The major site of virus replication appears to be in the peripheral lymphoid organs, such as the lymph nodes and the spleen, and in the mucosal lymphoid

tissue (Pantaleo et al., 1991, 1993; Embretson et al., 1993; Frankel et al., 1996; Veazey et al., 1998). Pivotal early studies of lymph node biopsies from infected individuals demonstrated that the fraction of lymphoid cells that were infected with HIV-1 was higher in the lymph nodes than in the peripheral blood. This finding, together with the fact that the vast majority (98%) of lymphocytes are located in the peripheral lymphoid tissues, has given rise to the idea that these tissues are the major site of virus replication. Some of the virus produced within the lymph nodes is trapped on a specialized cell type known as a follicular dendritic cell (FDC), which is distinct from the bone marrow–derived dendritic cell described above (Pantaleo et al., 1993). FDCs are located in the germinal centers of the secondary lymphoid organs where they function to bind and present intact antigens to B lymphocytes. FDCs express Fcγ and complement receptors that facilitate the trapping of immune complexes. In infected individuals, virus particles that have reacted with anti-envelope antibodies may be trapped on FDCs creating a major potential reservoir. There is some evidence for retention of infectivity despite the coating of the virion with antibody and complement that is necessary for attachment to FDCs (Heath et al., 1995). The FDC network is disrupted late in the course of disease at a time when the concentration of plasma virus rises (Pantaleo et al., 1993). Increases in plasma virus may reflect the loss of the trapping function of FDC. Virions that are produced in the lymph nodes and fail to bind to FDC can escape and enter the circulation.

To summarize, the prolonged asymptomatic phase of HIV-1 infection is characterized by a steady state in which continuous virus production is balanced by virus clearance. Most of the plasma virus is produced by infected $CD4^+$ lymphoblasts in the peripheral lymphoid tissues. Mechanisms of virus clearance remain unclear. A much better understanding of the dynamics of HIV-1 infection was achieved when antiretroviral drugs were used to perturb this steady state.

The First Phase of Decay

In 1995 the laboratories of George Shaw at the University of Alabama and David Ho at the Aaron Diamond AIDS Research Center published profoundly influential papers describing the effects of potent new inhibitors of HIV-1 reverse transcriptase and protease on plasma viremia in HIV-1-infected patients (Wei et al., 1995; Ho et al., 1995). These papers not only changed the course of AIDS therapy but also provided critical insights into the pathogenesis of the disease. The critical finding was that potent antiretroviral drugs produced a very rapid exponential drop in the level of plasma virus. Typically, plasma virus levels decreased 100-fold in two weeks (Figure 2). Analysis of this rapid decline led to the conclusion that HIV-1 infection is a dynamic process characterized by continual new rounds of viral infection of and replication in susceptible cells. Most of the plasma virus is produced by recently infected cells. Neither the free virus nor the cells that produce most of the plasma virus survive very long. It is important to recall that both the reverse transcriptase inhibitors and the protease inhibitors act to prevent new infection of susceptible cells

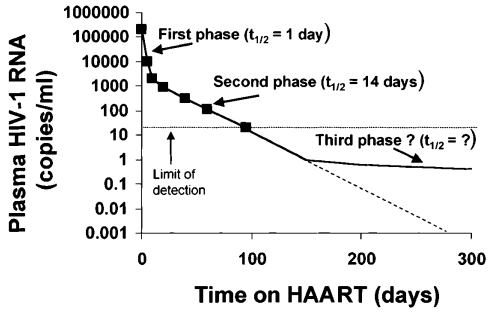

Figure 2. Hypothetical Decay Curve for Plasma Virus Levels in a Patient Treated with Highly Active Antiretroviral Therapy (HAART)

The $t_{1/2}$ for the first phase of decay is based in the work of Shaw and Ho and their colleagues (Wei et al., 1995; Ho et al., 1995; Perelson et al., 1996, 1997a). The $t_{1/2}$ of the second phase of decay is based on the work of Perelson et al. (1997a). The existence of a putative third phase of decay, reflecting the extremely slow turnover of a small number of latently infected resting $CD4^+$ T cells, is suggested.

without preventing virus production by cells that have already been infected. The rapid, exponential decline observed in treated patients was taken as evidence that the drugs largely prevent any new infection of susceptible cells. In this situation, the observed decreases in plasma virus levels reflect the intrinsic decay rates of the various cellular and extracellular compartments that harbor virus.

In a subsequent study, Perelson and colleagues examined plasma viral levels early after the initiation of therapy in an effort to measure separately the two processes that contribute to the rapid initial decay of plasma virus: the clearance of free virions and the loss of the infected cells that produce most of the plasma virus (Perelson et al., 1996). This was done by solving a set of differential equations describing the dynamics of cell infection and virion production and by fitting the decay data to the derived model. In this way, the decay rate constants for free virus (c) and for the cells that produce most of the plasma virus (δ) were calculated. The resulting values for c were strikingly similar in different patients, consistent with the idea that c is an intrinsic decay rate of a particular compartment. The same was true for δ. Mean values for c and δ were 3 and 0.5 day^{-1}, respectively. These rate constants are most easily evaluated in the form of half-lives ($t_{1/2} = \ln 2/c, \ln 2/\delta$). For free virus, $t_{1/2}$ was $<$6 hr. For productively infected cells, $t_{1/2}$ was $\sim$1–2 days. Direct confirmation of the rapid turnover of productively infected cells has been provided by Haase and colleagues using in situ hybridization analysis of tonsillar biopsies from patients starting antiretroviral therapy (Cavert et al., 1997). The lability of both the plasma virus and the cells that produce most of the plasma virus underscores the fact that virus replication is active and ongoing throughout the course of the disease. HIV-1 is cytopathic for $CD4^+$ T cells, and it is likely that the short-lived cells that produce most of the plasma virus are $CD4^+$ T lymphoblasts. A corollary is that long-lived infected cells such as macrophages and latently infected T cells must make only a minor contribution to plasma viremia in untreated patients.

Measurement of the viral clearance rate c allows calculation of P, the rate of virus production, using the

equation $P = cV$. Figures in excess of 10^{10} virions per day have been reported (Perelson et al., 1996). The high levels of virus produced per day are instructive in several regards (see Coffin, 1995). The high rate of virus production, when considered in the context of the short half-life of cells that produce most of the plasma virus, suggests that the rate of new infection of $CD4^+$ T cells is substantial. Uncertainty over the number of virions produced per infected cell (the burst size, N) makes it difficult to calculate precisely the number of productively infected cells (T*) from the steady-state relationship $N\delta T^* = cV$. However, Haase and colleagues have estimated T* by using in situ hybridization to detect cells expressing HIV-RNA in the lymphoid tissue (Haase et al., 1996). The total body value obtained, 4×10^7 cells, was in good agreement with estimates based on quantitation of integrated HIV-1 DNA in activated $CD4^+$ T cells in the lymph nodes (Chun et al., 1997a). Because of the technical difficulties involved, these approaches may underestimate the number of productively infected $CD4^+$ T cells. Nevertheless, it is clear that in an untreated patient, large numbers of $CD4^+$ T cells become infected daily. The reverse transcription process that occurs in each of those newly infected cells has a sufficiently high error rate that viral genomes with every possible single point mutation arise daily (Coffin, 1995). Perelson and colleagues have estimated that if 10^8 new cells are infected per day, then not only are all possible single point mutations generated daily, but almost 1% of all possible double mutations are generated each day (Perelson et al., 1997b). This finding has enormous implications for understanding the evolution of drug resistance mutations and viral escape from immune effector mechanisms.

The high rate of virus production also has implications for the debate over mechanisms of $CD4^+$ T cell depletion. Whether the direct killing of HIV-1-infected $CD4^+$ T cells is the cause of $CD4^+$ T cell depletion has been a central issue in AIDS research. The characteristics of the first phase of decay suggest that productively infected cells die quickly, but it has been unclear whether this effect alone is sufficient to account for $CD4^+$ T cell depletion. A major problem is the absolute number of $CD4^+$ T cells that die per day from infection is still unknown. The increase in $CD4^+$ T cell counts following initiation of HAART therapy is undoubtedly due in part to a decrease in virus-induced cell killing, but quantitative analysis of $CD4^+$ T cell reconstitution is complicated by the fact that early increases in CD4 counts may reflect a redistribution of $CD4^+$ T cells from the secondary lymphoid organs with a late component of increased production of naïve cells (Autran et al., 1997). Additional factors to be considered include the possibility that CD4 depletion is due to effects on thymopoeisis (Rabin et al., 1995; Su et al., 1995) or to indirect effects on uninfected $CD4^+$ T cells (Banda et al., 1992; Finkel et al., 1995). An excellent review of these complex issues has recently been published (Hellerstein and McCune, 1997).

The Second Phase of Decay

Although drug resistance developed rapidly in patients treated with protease inhibitors as monotherapy, further studies showed that combinations of antiretroviral agents could produce a decline in plasma virus to undetectable levels in many patients (Gulick et al., 1997; Perelson et al., 1997a). Careful studies by Perelson et al. showed that, after the rapid initial decay during the first 1–2 weeks of treatment, plasma virus declined at a slower rate, reflecting the turnover of a longer-lived viral reservoir or infected cell population (Figure 2). This reservoir accounts for only a small portion of the total virus production in an untreated individual and becomes evident only when the cells that produce most of the plasma virus have largely decayed. The half-life of the compartment responsible for this second phase of decay was estimated to be 1–4 weeks (Perelson et al., 1997a). A biphasic decay process was also observed directly in in situ hybridization studies of productively infected mononuclear cells in the lymphoid tissues (Cavert et al., 1997).

In many patients on highly active antiretroviral therapy (HAART), the second phase of decay brings levels of plasma virus down to below the limit of detection of current assays (20–500 copies/ml). Perelson et al. have used the careful analysis of second phase decay rates to make the first rational predictions of treatment times required for virus eradication, with the caveat that there may be undetected compartments or viral reservoirs that are not measurable by standard techniques (Perelson et al., 1997a). If it is assumed that there are no additional reservoirs, the second phase of decay extrapolates to zero residual infected cells in 2–3 years of completely suppressive antiretroviral therapy.

The nature of the viral reservoir that is responsible for producing most of the plasma virus during this second phase is unclear. One idea is that this virus is derived from infected macrophages that turn over much more slowly than infected $CD4^+$ lymphoblasts (Perelson et al., 1997a). The observed decay rate is compatible with measured rates for the turnover of macrophages in uninfected individuals. It remains unclear whether the number of macrophages infected in vivo is sufficient to account for the requisite level of virus production during the second phase. Virions trapped on FDC also decline in this time scale in individuals treated with HAART (Cavert et al., 1997).

The Third Phase of Decay

Despite the dramatic success of HAART in reducing plasma viremia to undetectable levels in some patients, there has been the concern that the infection will be "rekindled" if therapy is withdrawn. In patients who experience complete suppression of new cycles of replication for the estimated 2–3 years required for the second phase of decay to be complete, the residual virus that would rekindle the infection could only come from a compartment with the following two characteristics. First, this compartment would have to be extremely stable. Second, it would have to be responsible for producing only a minute fraction of the plasma virus in untreated individuals since the cells that produce most of the plasma virus decay quickly. As shown in Figure 2, the existence of such a compartment cannot be ascertained from the measurement of plasma HIV-1 RNA levels because of the difficulties involved in quantitating extremely low levels of plasma virus. Therefore alternative

approaches are needed to demonstrate the existence of compartments responsible for a putative third phase of decay.

Several recent studies suggest that latently infected resting memory CD4$^+$ T cells with integrated provirus represent an extremely stable reservoir for HIV-1 that may be responsible for the third phase of decay in patients on HAART (Chun et al., 1995, 1997a, 1997b; Finzi et al., 1997; Wong et al., 1997b). The importance of this reservoir derives from a fundamental aspect of the biology of memory cells; these cells must survive for long periods of time in order to provide protection against previously encountered pathogens. Although it has been presumed that the integration of HIV-1 DNA into the genomes of infected CD4$^+$ T lymphocytes allows viral persistence, there has been until recently little direct evidence that resting memory CD4$^+$ T cells with integrated provirus function as a latent reservoir for HIV-1 in infected individuals. Transformed cell lines carrying integrated HIV-1 DNA have been used to model latent infection (Folks et al., 1989; Pomerantz et al., 1990) and have provided a great deal of information about the molecular mechanisms involved in the regulation of HIV-1 gene expression. Direct evidence of proviral latency in vivo came from inverse PCR studies showing the presence of integrated HIV-1 DNA in extremely pure populations of resting CD4$^+$ T cells isolated from infected donors (Chun et al., 1995). The frequencies of such cells with integrated HIV-1 DNA were extremely low ($<$0.05%) and were similar in blood and lymph nodes, consistent with the continual recirculation of memory cells. The frequencies did not correlate with CD4 count, plasma RNA, or therapy (Chun et al., 1997a). Those findings suggested that, in asymptomatic HIV-1 infection, a relatively stable systemic steady state is established in which only a minute fraction of the resting CD4$^+$ T cell population carries integrated HIV-1 DNA (Coffin, 1995; Chun et al., 1997a). The total body number of resting CD4$^+$ T cells with integrated HIV-1 DNA was estimated to be approximately 10^7 cells, only a fraction of which carry replication-competent provirus. Among resting CD4$^+$ T cells, integrated HIV-1 DNA was present primarily among cells with a CD45RO$^+$ phenotype, characteristic of memory cells (Chun et al., 1997a).

Although several studies have noted that amplifiable HIV-1 DNA persists in the cells of patients on long-term HAART (Perelson et al., 1997a; Cavert et al., 1997; Wong et al., 1997a), it has often been presumed that the proviral DNA burden in T cells represents mainly defective archival sequences. In fact, it is often difficult to isolate HIV-1 from T cells of patients on long-term HAART using conventional virus culture methods. However, with enhanced virus culture conditions designed to induce uniform activation of resting CD4$^+$ T cells, replication-competent virus can be recovered from highly purified resting CD4$^+$ T cells (Chun et al., 1997a). The frequencies of resting CD4$^+$ T cells with replication-competent provirus are lower than the frequencies of resting CD4$^+$ T cells with integrated HIV-1 DNA (as detected by inverse PCR). This suggests that some of the integrated HIV-1 DNA in resting CD4$^+$ T cells is defective. Nonetheless, the finding that replication-competent virus can persist in a latent form in resting CD4$^+$ T cells raises the possibility that this reservoir might represent a major barrier to virus eradication in patients on HAART.

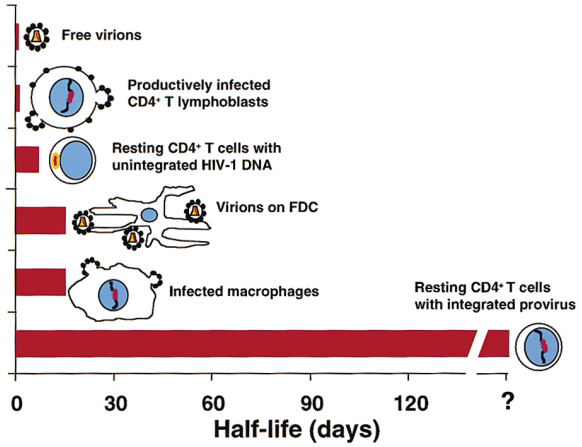

Figure 3. Estimated Half-Lives of Cellular and Extracellular Compartments Harboring HIV-1

The half-life of free virions is $<$6 hr, based on the work of Perelson et al. (1996). The half-life of the cells that produce most of the plasma virus was measured by Wei et al. (1995) and by Ho and colleagues (Ho et al., 1995; Perelson et al., 1996, 1997a). The most recent estimates suggest a half-life of 1 day. The stability of the unintegrated HIV-1 DNA in resting CD4$^+$ T cells is still uncertain. Rescue of infectious virus can be achieved by activation of the cells, and some studies have noted a loss in the ability to rescue virus after several days in culture (Zack et al., 1988; Chun et al., 1997a). Failure to rescue infectious virus from infected resting cells could reflect degradation of unintegrated HIV-1 DNA or loss of some essential protein components of the preintegration complex. In other studies, the intermediates that formed following in vitro infection of resting CD4$^+$ T cells showed greater stability (Spina et al., 1995). Analysis of this issue is complicated by the fact that resting CD4$^+$ T cells do not survive in vitro for more than 2–3 weeks. The pool of extracellular virions bound to FDC shows a biphasic decay with a rapid initial phase ($t_{1/2}$ = 1.7 days) followed by a second slower phase of decay ($t_{1/2}$ = 14 days) (Cavert et al., 1997). The half-life of infected macrophages remains a critical unknown. Perelson et al. (1997a) have suggested that these cells may be responsible for the second phase of decay ($t_{1/2}$ = 14 days). This rate of turnover would be consistent with that observed for macrophages in uninfected individuals (Van Furth, 1989). However, it must be kept in mind that the rate of macrophage turnover may depend upon the tissue and may be influenced by HIV-1 infection. Finally, the rate of turnover of latently infected resting CD4$^+$ T cells with integrated HIV-1 DNA is unknown. In initial cross-sectional studies of patients on HAART, no significant decay is apparent in the first 2 years of therapy (Finzi et al., 1997). For theoretical reasons discussed in the text, it is possible that the half-life of this compartment may be even longer than that average half-life of resting memory CD4$^+$ T cells. In uninfected individuals, these cells have a mean intermitotic interval of 5.5 months (McLean and Michie, 1995).

Three recent studies examined whether replication-competent virus could persist in the resting CD4$^+$ T cells of patients on HAART (Finzi et al., 1997; Wong et al., 1997b; Chun et al., 1997b). These studies focused on a subset of patients who responded extremely well to therapy and who had been aviremic for as long as 2.5 years. Although it is generally very difficult to isolate virus from patients on long term HAART by conventional methods, all three groups found that with enhanced culture techniques, replication-competent virus could

be readily isolated from resting $CD4^+$ lymphocytes of these patients. Most importantly, in cross-sectional analysis, the frequencies of latently infected $CD4^+$ T cells showed no detectable decrease during the first two years of therapy (Finzi et al., 1997). This is in marked contrast to other viral reservoirs examined to date, all of which show readily measurable decay rates (Figure 3). These results suggest an extremely slow decay rate for this compartment, consistent with the long-term survival of resting memory $CD4^+$ T cells in uninfected individuals (mean intermitotic interval = 5.5 months; McLean and Michie, 1995). At the present time, it is unclear whether the extraordinary stability of this compartment is due to the long life span of latently infected memory cells or to replenishment of the compartment by very low levels of ongoing viral replication. Sequence analysis of viruses isolated from this compartment revealed little evidence for the evolution of drug resistance, suggesting that the viruses that persist in this compartment are derived from long-lived cells infected prior to the initiation of HAART (Finzi et al., 1997; Wong et al., 1997b). One critical factor in determining how long a latently infected memory cell will persist in this compartment is the frequency with which the relevant antigen is encountered. Latently infected $CD4^+$ T cells specific for frequently encountered antigens may be deleted from the reservoir, leaving only cells specific for rarely encountered antigens. It is therefore possible that the half-life of this compartment may be even longer than the mean half-life of memory T cells in uninfected individuals.

Taken together, these results suggest that HIV-1 can establish latent infection in long-lived resting memory $CD4^+$ T cells and that this reservoir may represent a major barrier that will have to be overcome in efforts to eradicate the infection with antiretroviral therapy. More optimistically, it is important to note that the compartment is comprised of fewer than 10^7 cells. It is possible that enhancement or reconstitution of immune responses to HIV-1 in treated patients, including $CD4^+$ helper responses, could eventually allow immunologic control of viruses originating from this reservoir (Rosenberg et al., 1997).

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